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APPLICATION NUMBER: *60/533,745*
FILING DATE: *December 30, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/43969*

Certified by

Jon W. Dudas

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PROVISIONAL APPLICATION COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION Under 37 CFR 1.53 (b)(2).

Attorney Docket No.	588.P	Type a plus sign (+) inside this box -----> +

INVENTOR(s)/APPLICANT(s)

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (CITY AND EITHER STATE OR FOREIGN COUNTRY)
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TITLE OF THE INVENTION (280 characters max)

Efficacy of PMEG [9-(2-phosphonylmethoxyethyl)guanine] and its prodrug cPr-PMEDAP [9-(2-phosphonylmethoxyethyl)-N6-cyclopropyl-2,6-diaminopurine in organotypic cultures of normal and papillomavirus ((HPV)-positive cells

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ENCLOSED APPLICATION PARTS (check all that apply)

- [X] Specification — *Number of pages* 1
- [ ] Small Entity Statement
- [ ] Drawing(s) — *Number of sheets* _____
- [ ] Other (specify)

METHOD OF PAYMENT (check one)

[X] The Commissioner is hereby authorized to charge filing fees (as well as any additional fees which may be required by this paper) and credit Deposit Account Number **07-1250.**

Provisional Filing Fee Amount ($) **$160.00**

The invention was made by an agency of the United States Government of under a contract with an agency of the United States Government.

- [X] No.
- [ ] Yes, the name of the U.S. Government Agency and the Government contract number are:

Respectfully submitted,

SIGNATURE _____ DATE December 30, 2003

TYPED or PRINTED NAME Mark Bosse REGISTRATION NO. (if appropriate) 35,071

- [ ] Additional inventors are being named on separately numbered sheets attached hereto

PATENT

Attorney Docket No. 588.P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: William A. Lee, et al

For: Efficacy of PMEG [9-(2-phosphonylmethoxyethyl)guanine] and its prodrug cPr-PMEDAP [9-(2- phosphonylmethoxyethyl)-N6-cyclopropyl-2,6-diaminopurine in organotypic cultures of normal and papillomavirus ((HPV)-positive cells

Mail Stop Provisional Patent Application
Commissioner for Patents
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Alexandria, VA 22313-1450

PROVISIONAL APPLICATION COVER SHEET
(37 C.F.R. § 1.51 (2) (i))

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this "Provisional Application Cover Sheet" and the documents referred to as attached therein are being deposited with the United States Postal Service on this date December 30, 2003 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number ER622365239US addressed to the: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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Efficacy of PMEG [9-(2-phosphonylmethoxyethyl)guanine] and its prodrug cPr-PMEDAP [9-(2- phosphonylmethoxyethyl)-N6-cyclopropyl-2,6-diaminopurine in organotypic cultures of normal and papillomavirus ((HPV)-positive cells

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We have recently developed organotypic co-cultures of primary human keratinocytes (PHKs) isolated from neonatal foreskins and the cervical carcinoma cell line SiHa (HPV-16 positive) to evaluate the selectivity of cidofovir, an acyclic nucleoside phosphonate analogue (ANPs) that proved efficacious in the treatment of different manifestations of HPV-induced epithelial cell proliferation. We have now used this system to determine the efficacy and selectivity of other ANPs with potential activity against HPV, PMEG and cPr-PMEDAP. The organotypic raft culture permits cells to proliferate and fully differentiate at the air-liquid interface on a dermal-equivalent support. Normal keratinocytes stratify and fully differentiate in a manner similar to the normal squamous epithelial tissues, while HPV-positive cell lines exhibit dysplastic morphologies similar to (pre)neoplastic lesions seen *in vivo*. SiHa cells and normal PHKs were seeded at a 1:1 ratio on top of the dermal equivalent and maintained submerged for 48 h. The collagen rafts were raised (day 0) and placed on satinless-steel grids, at the interface between air and liquid culture medium. Epithelial cells were then allowed to proliferate for 10 days. At different times after lifting the rafts different concentrations of the compounds were added. After 10 days all cultures were fixed, paraffin-embedded, sectioned and stained with hematoxylin and eosin. In control untreated co-cultures, rafts showed regions with dysplastic morphology, normal epithelium and areas with mixtures of both types. In contrast, rafts that were treated with PMEG 0.5 μg/ml and cPr-PMEDAP 5 μg/ml at day 3 post-lifting and with PMEG 0.5 and 5 μg/ml and cPr-PMEDAP 5 μg/ml at day 6 post-lifting showed areas of fully differentiated normal epithelium and absence of the tumor cells. Inhibition of SiHa cell proliferation in the raft co-cultures by both drugs was concentration- and time-dependent. These results point to a selective mechanism of inhibition of HPV-positive cell growth by PMEG and cPr-PMEDAP compared to PHKs.